REMARKS

Applicants have amended claim 18 preamble to refer to identification of a "gene mutation" instead of "gene." Step b) has been amended to include the term "mutant" to refer back to step a). These amendments are supported by the specification as a whole and particularly, for example, on page 3, lines 4-7; par. bridging pp. 4 and 5; page 14, lines 1-5; and page 17, lines 6-8. Therefore, applicants submit that the amendments are supported by the specification and do not introduce new matter and their entry is respectfully requested.

Step d) has been amended to include the phrase "is determined to harbor" to more clearly point out that the purpose of step d) is to sort out the gene mutations that are specifically involved in cell proliferation. The amendment is supported specifically, for example, on page 4, lines 1-3. Therefore, the amendment does not introduce new matter and its entry is respectfully requested.

Step e) has been amended to refer to identification of the "gene mutation in the mutant fish as being involved in carcinogenesis". This amendment is supported specifically, for example, on paragraph bridging pages 3 and 4 and on page 13, last paragraph and page 14, first paragraph.

Accordingly, the present amendments are supported by the specification and do not introduce new matter and their entry is respectfully requested. Applicants believe that the amendments to the claims clarify the method and at minimum reduce the issues for the appeal.

Turning now to the specific rejections by the examiner.

Claim Rejections Under 35 USC § 112

Claims 18-29 were rejected under 35 USC § 112.

The method of the invention is directed to identification of gene mutations that are specifically involved in carcinogenesis without the necessity to first clone genes and then determine whether mutations in them are involved in carcinogenesis.

Applicants believe that the amendments to claim 18 as described above address Examiner's concerns about the essential steps involved in identification of gene mutations associated with carcinogenesis, and therefore obviate the rejection. Therefore withdrawal of the rejection under 35 USC § 112 is respectfully requested. At minimum, the amendments should reduce the issues on appeal.

Claim Rejections Under 35 USC § 103(a)

The present invention is to a particular method of indicating mutations related to cell proliferation defects without the necessity of first cloning the genes by using zebrafish. The method of the present invention comprises particular steps a-g. The examiner cites the following quotes from the prior art to argue that one skilled in the art would have been motivated **to combine the teachings of four or even five different references** to come up with the combination of steps which describes the method of the present invention:

Spitsbergen: "Zebrafish offer a cost-effective, relatively rapid system in which to address questions regarding cellular and molecular mechanisms in carcinogenesis."

Cheng: "The increasingly powerful genetic and experimental tools available for work with zebrafish can be used to address a broad range of questions in vertebrate biology."

"It is also important to point out that the experimental features that make zebrafish so useful in dissecting the mysteries of the development can be applied to the elucidation of other significant problems in vertebrate biology."

Driever: "Of course, its [zebrafish] contributions can only be enhanced, once the genes are cloned, by extrapolation to mouse, by targeted gene ablation, and to human, by assessment of linkage to common disorders."

In light of the Applicant's disclosure, it is easy to argue that a general statement in a developmental biology-related article "other significant problems in vertebrate biology" refers to "carcinogenesis."

Likewise, in light of the Applicant's disclosure, it is easy to argue that a general statement in a developmental biology-related article "a broad range of questions in vertebrate biology" refers to "carcinogenesis."

Moreover, in light of the Applicant's disclosure, it is easy to argue that a general statement in a developmental biology-related article "common disorders" refers to "carcinogenesis."

Therefore, Applicants submit that deriving motivation to combine teachings of all these different references to come up with the 7-step method of applicant's invention based upon these general statements provide a classic example of hindsight reasoning.

Applicants respectfully submit that the only teaching in Spitsbergen is that zebrafish do develop tumors. However, there is no suggestion whatsoever in Spitsbergen to combine it with any of the secondary references to come up with any kind of a method to utilize zebrafish to actually "address questions regarding cellular and molecular mechanisms in carcinogenesis."

Therefore, Applicants submit that without Applicant's disclosure, one would not be motivated to collect the cited secondary references in the field of developmental biology, to come up with even a partial method to identify carcinogenesis-related mutations in the fish.

Moreover, as described below in detail, even a combination of the cited references does not teach all the steps the particular method of the present invention.

Specifically:

Claims 18-21, 23, and 29 were rejected under 35 USC § 103(a) as being obvious over Spitsbergen in view of **three different** secondary references including Driver, Cheng and Alexander.

The method of Claim 18, begins with step a) "wherein the fish are exposed to a mutagen". This step is the only teaching a skilled artisan can rely Spitsbergen for.

The Examiner argues, that Spitsbergen also teaches steps f) and g) of claim 18. Applicants strongly disagree. Step f) of Claim 18 requires that the fish be a mutant fish mated with a wildtype fish, i.e. an F2 generation fish, that is exposed to the carcinogen in parallel with a

wildtype fish. Nowhere in Spitsbergen is there a teaching of crossing mutant fish with a wild-type fish and then re-exposing the resulting F2 generation fish to a carcinogenic substance in parallel with a wildtype fish. Similarly, there is no mention, explicit or even implicit, of step g) which requires comparing the frequency of the tumor formation between a wildtype fish exposed to carcinogen and a mutant fish exposed to carcinogen to identify a gene(s) involved in mutagenesis by observing accelerated tumor formation by comparing tumor formation in a wild-type fish and mutant fish.

Therefore, all Spistbergen teaches is that wild-type zebrafish embryos and fry when exposed to MNNG produce tumors in the adult fish and that the fish can therefore be used in carcinogenesis studies. How this could be accomplished is not even discussed. The Examiner tries to argue that the method of the present invention would have been obvious had this very general teaching been combined with three separate references, but fails to even show that all elements of the method are taught by these numerous references. Furthermore, the Examiner also fails to show that there is either an explicit or implicit suggestion to combine these specific references, or that a skilled artisan would have been motivated to combine the references to derive the method of the present invention.

First, the Examiner must show that there is suggestion and motivation to combine the secondary references with the primary reference. Second, the examiner must show that if combined, these references teach all the steps of the present method. General implications in light of applicant's teachings are not enough, the requisite motivation to combine the references must come from the prior art . See, e.g., *In re Dow Chem. Co. v. American Cyanamid Co.,* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531-32 (Fed. Cir. 1988)("[t]here must be a reason or suggestion in the art for selecting the procedure used, other than the knowledge learned from the applicant's disclosure).

The Examiner relies upon Driever to show the "general state of the art." (p. 6) The Examiner concludes that "[t]he outline reviewed by *Driever et al* illustrated the state of the art in using zebrafish for investigating genes important in vertebrate development and readily

applicable for investigating mutagens and carcinogenesis." (page 6, emphasis added) Driever does not explicitly suggest using the claimed method to identify specific cancer related genes without cloning the genes first using zebrafish. The Examiner tries to argue that such teaching can be implied from statements such as the last sentence of Driever. The last paragraph reads

> "It may be the power of the zebrafish, in terms of generation of new paradigms for development, is precisely with regard to vertebrate features, such as organ assembly. Of course, its contributions can only be enhanced, once the genes are cloned, by extrapolation to mouse, by targeted gene ablation, and to human, by assessment of linkage to common disorders." (emphasis added)

Applicants respectfully submit that all that can be implied from this paragraph is that once zebrafish genes are cloned, their function may be correlated to 'common disorders', for example in human. Even if one were to argue that the reference to 'common disorders' implies 'cancer', the premise and method of the present invention is exactly to the contrary what is discussed in this paragraph. The present claims are directed to a method to first create a random cell proliferation defect without cloning it first and then fish out the gene responsible for it by only comparing the tumor formation in the mutant fish to that of a wild-type fish, both exposed to a known carcinogen and then comparing the frequency of tumor formation between them wherein accelerated tumor formation indicates that the mutant fish contains a mutation responsible for a cell proliferation defect. This method identifies the association between a defect and the phenotype before cloning the specific gene.

The Examiner relied upon Cheng and Alexander "to show details of using zebrafish in uniparental and two-generation screening." Both of these references deal with identification of genes important in vertebrate development. Further, neither Cheng nor Alexander teach comparison of tumor formation frequency of mutagen exposed wildtype fish to that of a mutagen exposed mutant fish, and identifying the carcinogenesis associated gene solely on the basis of accelerated tumor formation without first having to clone the gene. Therefore, even assuming, *arguendo*, that there would be a suggestion and motivation to select and combine these two

references with Spitsbergen in light of Cheng, even the combination would not teach all the steps of the present invention.

In light of the above, the **three cited secondary references,** even in combination with Spitsbergen, do not teach all the steps of the claimed method. Therefore, applicants submit that the rejection of claims 18-21, 23, and 29 under 35 USC § 103(a) over Spitsbergen in view of Driver, Cheng and Alexander is improper and should be withdrawn.

Claims 18-24, and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of a **fifth reference,** Epstein.

Applicants strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Epstein merely teaches antisense oligonucleotides and their use as cell proliferation markers but does not in any way teach or suggest use of such probes in a zebrafish nor does Epstein teach a screen for novel carcinogenesis associated genes using zebrafish haploid screen followed by carcinogenesis assay. Epstein does not teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first. Therefore, Epstein does not overcome the deficiency in Spitsbergen, Driever, Cheng and Alexander as described above, the discussion of which is incorporated herein by reference. Consequently, applicants submit that the rejection of claims 18-24, and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng, and Alexander in view of Epstein is improper and should be withdrawn.

Claim 25 was rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of Vogelstein et al. (U.S. Patent No. 6,511,818).

Vogelstein does not teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first. Therefore, Vogelstein does not overcome the deficiency in Spitsbergen, Driever, Cheng and Alexander as described above, the discussion of which is incorporated herein by reference. Consequently,

applicants submit that the rejection of claims 25 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng and Alexander in view of Vogelstein is improper and should be withdrawn.

Claims 18-21, 23, 25, and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of Shyjan.

Applicants strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Shyjan teaches a method of using cell proliferation markers in the flowcytometric assay. There is, however, no teaching or suggestion that such method could be used in a double screening wherein mutant fish embryos are first screened for cell proliferation defects and such defective mutants be consequently screened in a carcinogenesis assay to show whether the cell proliferation defects are associated with carcinogenesis. Furthermore, Shyjan does not teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first. Therefore, Shyjan does not overcome the deficiencies in the four other cited references. And again, the examiner has provided no indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness. Consequently, applicants submit that the rejection of claims 18-21, 23, 26, 27 and 29 under 35 USC § 103(a) over of Spitsbergen, Driever, Cheng, and Alexander in view of Shyjan is improper and should be withdrawn.

Claims 18-21, 23, 26, 27 and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of O'Reilly.

Applicants strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Like teachings of Epstein and Shyjan, wherein only specific marker or method, respectively, are taught, O'Reilly only teaches TUNEL as a method for identifying cell proliferation defects. And again, O'Reilly does not teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and

mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first. Thus, O'Reilly does not overcome the deficiencies in the four other cited references. Like for the arguments discussed above, the examiner also here has provided no indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness.

Therefore, applicants submit that the rejection of claims 18-21, 23, 26, 27 and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng and Alexander in view of O'Reilly is improper and should be withdrawn.

Claims 18-21, 23, 28 and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of Li.

Applicants again strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Li teaches that BrdU stain can be used as a diagnostic marker for tumors but this teaching does not overcome the deficiencies in the four other cited references. Li does not teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first. Therefore, as discussed above, Li does not overcome the deficiencies in the other three references. Once again, the examiner fails to provide any indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness. Therefore, applicants submit that the rejection of claims 18-21, 23, 28 and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng and Alexander in view of Li is improper and should be withdrawn.

In conclusion, one skilled in the art, without knowledge of the present invention and thus without the benefit of impermissible hindsight, would not have been motivated to combine the primary reference discussing carcinogenic properties of MNNG on zebrafish **with the three or**

even four additional secondary references discussing use of zebrafish in studying vertebrae development to arrive at the method of the claimed invention. Furthermore, even assuming, *arguendo,* that one were to combine the references, the combination would not teach or suggest all the steps of the present invention as discussed above because none of the cited references teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first.

In view of the foregoing, applicant respectfully submit that all claims are now in condition for allowance. At minimum, the presented claim amendments should reduce the issues on appeal.

In the event that any additional fees are required, the Commissioner is authorized to charge Nixon Peabody deposit account No. 50-0850 for fee deficiencies associated with this submission.

Respectfully submitted,

Date: 1/20/04

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